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Phenotypic Variability of Environmental Isolates of the Entomopathogenic Fungus *Beauveria bassiana*

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Abstract—A total of 35 isolates of *Beauveria bassiana* (Bals.) Vuill. isolates obtained from various insects of Novosibirsk oblast were investigated. The fungal morphotypes were found to include cultures of high, medium, and low virulence. Low correlation ($r < 0.48$) was observed between virulence and the morphophysiological characteristics of the isolates (lipase and protease activity, biomass, radial growth rate, conidia productivity, and relief). Isolates exhibiting high virulence to insects of a certain order proved to be virulent to the insects of other orders. A high correlation ($r > 0.74$) was revealed between the virulence of the isolates to the potato beetle *Leptinotarsa decemlineata* Say and the locusts *Calliptamus barbarus* Costa and *Locusta migratoria* L. Isolates obtained from insects of the same species in the same site may differ significantly in virulence.

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The entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. attacks diverse insects of various orders. The strains of this species exhibit significant differences in morphophysiological characteristics and virulence. In the present paper, virulence is treated as a qualitative measure of pathogenicity, in accordance with the literature on insect pathology [1]. Geographical isolation is known to play the main role in the intraspecific differentiation of *B. bassiana* [2]; however, its intrapopulation variability is still poorly studied. A number of authors had isolated *B. bassiana* morphotypes and established the relations between colony morphology, sporulation intensity, and virulence [3–6]. The strains with floury smooth colonies were found to be the most virulent and promising for mass cultivation, while the cultures with felted or cottonlike structure proved to be less virulent and weakly sporulating. Although some works demonstrated a relation between the virulence of *B. bassiana* strains and activity of their proteolytic, lipolytic, and chitinolytic enzymes [7, 5], such a correlation was not found by other authors [8]. The issue of association of *B. bassiana* strains with a certain group of hosts is still not settled. It is not known whether the virulence of a fungal strain depends on the taxonomic position of the host from which it had been isolated. Some authors believe that adaptation to the local insect populations may result in formation of the fungal races with high specificity to certain hosts [6, 9]. However, within the genus *Beauveria* association with specific groups of insects can be found only at the species level and is not distinct [2]. In other broadly specialized filamentous fungi, such as *Metarhizium anisopliae* (Metsch.) Sor., intraspecific specialization for the insects of different orders [10] or species [11] was established. Investigation of polymorphism and trophic preferences of *B. bassiana* is important for practical ecology, especially for development of the programs for artificial epizootics in insect populations.

The goal of the present work was investigation of the morphophysiological and virulent characteristics of *B. bassiana* isolates obtained from different groups of insects in a limited area (Novosibirsk oblast).

MATERIALS AND METHODS

Most of the *B. bassiana* isolates (33) were obtained from various insects in eastern Novosibirsk oblast (54°35′–55°05′ N, 82°45′–84°00′ E) from July to September 2006. Two strains (Cap-31 and BBK-1) were isolated in 2000–2001 in southern Novosibirsk oblast near Karasuk (53°03′ N, 78°03′ E). The insect hosts belonged to the following taxa: *Hetroptera: Miridae* sp. (2), *Pentatomidae* sp. (2); *Orthoptera: Calliptamus italicus* L. (2); *Homoptera: Cicadidae* sp. (1); *Coleoptera: Brachysomus echinatus* Bonsd. (9), *Polydrusus undatus* F. (1), *Eudipnus mollis* Strom (1), *Staphylinidae* sp. (2), *Cisidae* sp. (1), *Chrysomelydae* sp. (1), *Carabidae* sp. (1), *Elateridae* sp. (1); *Lepidoptera: Thyatiridae* sp. (1), *Macroheterocera* sp. (3); *Hymenoptera: Cimbex* sp. (1), *Formicoidea* sp. (2); *Diptera: Asellidae* sp. (1), *Cyclorrhapha* sp. (1); and *Insecta* sp. (2). Isolation and maintenance of the cul-

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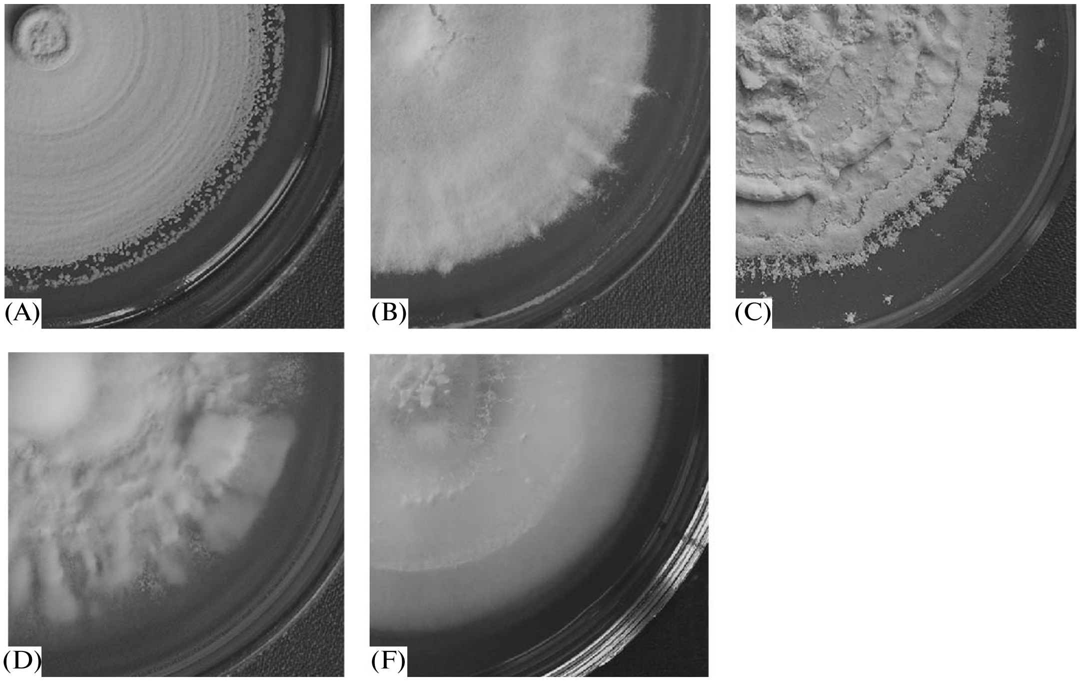

Fig. 1. Morphological variants of *B. bassiana* on agarized Waxman medium: floury, without relief (A); felted, without relief (B); floury–felted, with relief (C); felted, with relief (D); and cottonlike (F).

tures were carried out according to accepted techniques [12].

The biomass of aerial mycelium and the number of spores were determined by lawn plating on agarized Waxman's medium [13] in 90-mm petri dishes (64 cm^2). After 45 days of cultivation at 25°C, the aerial mycelium and spores were removed with a spreading rod, dried for 10 days at the same temperature, weighed, and homogenized in a mortar. Conidia were counted in a Goryaev chamber.

The rate of fungal growth was determined by point-inoculation of the Waxman's and Sabouraud's agarized media with subsequent measurement of the colony diameter every other day for a month. The colony relief was assayed visually on the 30th day as follows: no relief (1), weak relief (2), and pronounced relief (3).

Proteolytic and lipolytic activity was determined by an express method on agarized media supplemented with dry skim milk (2%) or Tween-80 [7]. Agar blocks (7 mm in diameter) with 3-day fungal mycelium were placed on these media. The width of proteolysis zones after 3 days of incubation and the width of lipolysis zones after 6 days of incubation at 25°C were used as indices of the enzymatic activity.

The virulence of the isolates was assayed on II and III instar larvae of the potato beetle (*Leptinotarsa decemlineata* Say), on II and III instar nymphs of the locusts *Calliptamus barbarus* Costa and *Locusta migratoria migratoria* L. (migratory locust), and in some cases on III instar larvae of small tortoiseshell (*Aglais urticae* L.). The insects were infected by single dipping into water suspensions of fungal conidia. The larvae were maintained in mesh-covered 700-ml plastic containers. The experiments were carried out in at least three repeats of 10–15 individual insects. Feed replacement and mortality assessment were carried out daily for 10–17 days, depending on mortality level.

For comparative analysis of virulence of the isolates, the mortality of the locust nymphs and small tortoiseshell caterpillars was determined on the 8th–10th day after infection and of the potato beetle on the 12th–15th day. The mortality level at this time exhibited the highest dispersion, allowing precise differentiation of the isolates by their virulence.

RESULTS

Cultivation of the isolates on the Waxman and Sabouraud media revealed five types of colonies (Fig. 1). The colonies of the first type were floury, without relief (A); of the second type, felted, without relief (B); of the third type, floury–felted, with relief (C); of the fourth type, felted, with relief (D); and of the fifth type, cottonlike (F). The morphological variants D (18 isolates) and B (8 isolates) were the most common ones; variants A, C, and F occurred more seldom (3 isolates each). The cultures with floury colony structure (A) produced the highest conidial titers biomass (Fig. 2). The lowest titer and biomass were observed in the morphological variants B and D. The variant A is also characterized by rapid development of

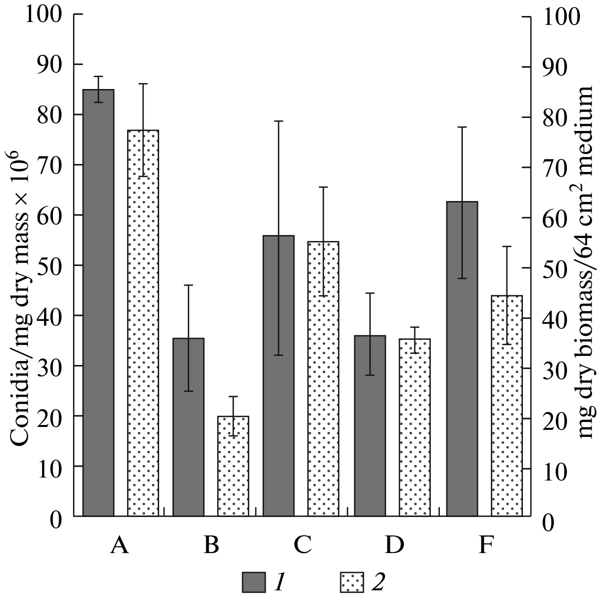

Fig. 2. Sporulation intensity and biomass yield in *B. bassiana* isolates from different morphological variants on Waxman medium: number of conidia (*1*) and biomass (*2*). The bars indicate errors of the mean. Designations of the variants are the same as on Fig. 1.

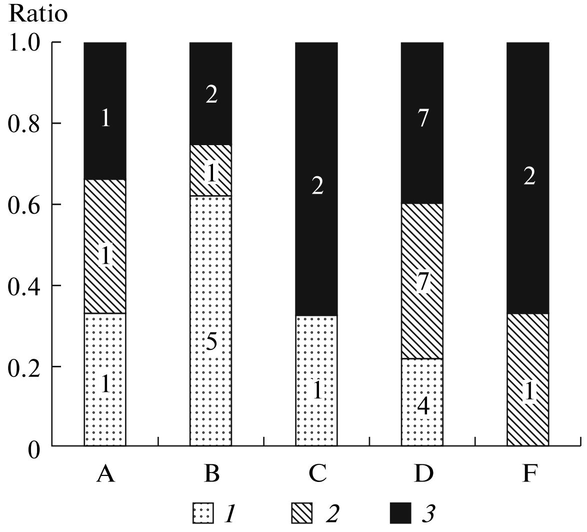

Fig. 3. The ratio of isolates with different levels of virulence to potato beetle among *B. bassiana* morphological variants (titer is 5 × 10^6 conidia/ml, 15th day after inoculation). Low virulence (10–40% mortality) (*1*), medium virulence (40–70%) (*2*), and high virulence (70–100%) (*3*). The numerals indicate numbers of isolates. Designations of the variants are the same as on Fig. 1.

sporulation on the 9th–13th day. Other variants developed spores mostly on the 20th–35th day.

Experiments on potato beetle larvae demonstrated that the isolates with low, medium, and high virulence may belong to any morphological variant; the ratio of highly virulent cultures was higher among the isolates with more pronounced colony relief (Fig. 3). Experiments with *Calliptamus barbarus* revealed a similar tendency.

All the isolates were found to possess proteolytic and lipolytic activity. The zones of proteolysis were 1–8 mm wide, and the zones of lipolysis were 16–26 mm wide. No reliable correlation was found between virulence and protease activity of the isolates ($r < 0.27$, $p > 0.05$) (Table 1). Neither was a relation found between virulence and the rate of radial colony growth on different media ($r < 0.14$, $p > 0.05$). A weak positive correlation of about 0.38–0.45 ($p < 0.05$) was found between lipase activity and virulence to the locusts and potato beetle. Reliable correlation at about 0.35–0.48 ($p < 0.05$) was revealed between conidia productivity, colony relief, and virulence to the tested insects.

At the first stage of the work, comparative assessment of virulence to different insects was carried out with two *B. bassiana* isolates, Cap-31 and BBK-1. These cultures have been tested on insects for four years (2005–2008). These isolates have highly stable cultural and virulent characteristics. They have been isolated from the same insect species (*C. italicus*) in the same site (vicinity of Karasuk) and have the same colony type (A). An isolate highly virulent to members of one order of insects was found to be highly active against insects from other orders. For example, BBK-1 caused higher mortality in migratory locust, potato beetle, and small tortoiseshell than Cap-31 (Fig. 4). Similar results were obtained by the authors earlier in investigation of two strains of another entomopathogenic fungus, *M. anisopliae* [14].

At the second stage of the research, all 35 environmental isolates of *B. bassiana* were investigated. A reliable close relation was established between virulence

Virulence of *Beauveria bassiana* isolates obtained from insects of various orders to the larvae of *Leptinotarsa decemlineata* and *Calliptamus barbarus*

Host insect (order)	Number of isolates	Mortality of *L. decemlineata*, %	Mortality of *C. barbarus*, %
Homoptera	1	93	100
Heteroptera	4	38 ± 9	740 ± 14
Orthoptera	2	44 ± 17	65 ± 20
Coleoptera	17	55 ± 6	71 ± 6
Lepidoptera	4	68 ± 14	84 ± 12
Hymenoptera	3	34 ± 8	57 ± 14
Diptera	2	25 ± 3	48 ± 15

Note: Inoculum concentration for infection of *L. decemlineata* was 5 × 10^6 conidia/ml (determination on the 15th day of experiment), for *C. barbarus*, 1 × 10^6 conidia/ml (determination on the eighth day of experiment). The error of mean is given after the "±" sign.

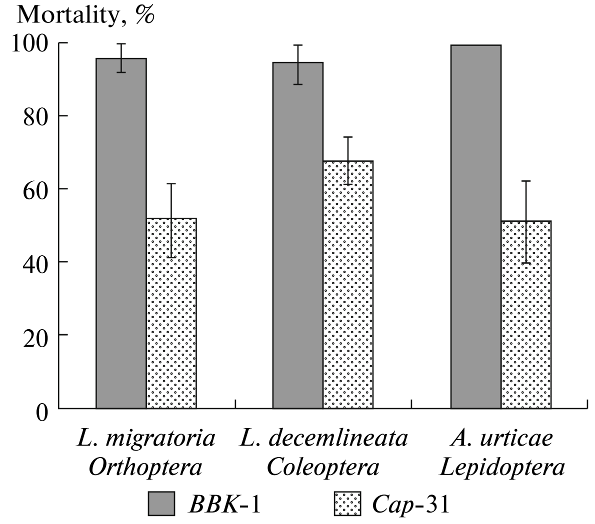

Fig. 4. Virulence of two *B. bassiana* isolates to insects of different orders (titer is 1×10^7 conidia/ml, 10th day for *Locusta migratoria* and *Aglais urticae* and 12th day for *Leptinotarsa decemlineata*).

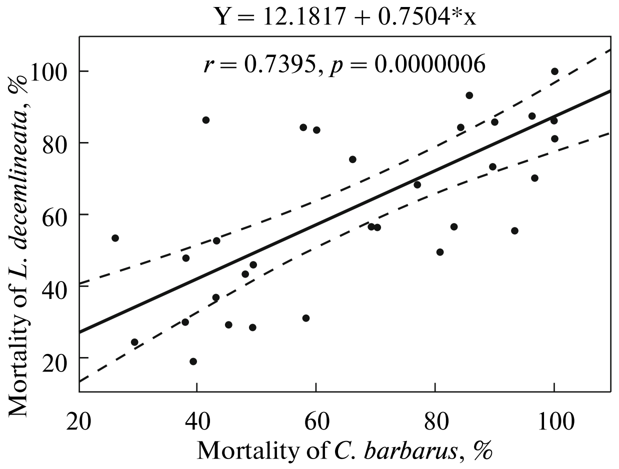

Fig. 5. Virulence of *B. bassiana* to the larvae of *L. decemlineata* (15th day, 5×10^6 conidia/ml) and *C. barbarus* (8th day, 1×10^6 conidia/ml).

of these cultures against *Calliptamus barbarus* and potato beetle (Fig. 5). This experiment was repeated on the larvae of migratory locust and potato beetle with a smaller collection of strains (26) In this case, the correlation was also strong and highly reliable ($r = 0.82$, $p < 0.00001$). This is probably an indication of the absence of specialization of these isolates in respect to the insects of these orders. The differences in virulence between the groups of isolates obtained from different orders of insects were not significant. High variability was observed in insect mortality (table). The strain isolated from a cicada was the most virulent to potato beetle and *Calliptamus barbarus*. The cultures isolated from lepidopterans and beetles were relatively virulent to both species; those isolated from hemipterans were toxic to *Calliptamus barbarus*. The cultures from orthopterous insects, dipterans, and hymenopterans were less virulent. The strains isolated from the same insect species in the same location may differ significantly in virulence. For example, eight isolates of *B. bassiana* obtained from the weevil *Brachysomus echinatus* in a small parcel of birch wood (100 m^2) exhibited highly varied virulence to potato beetle (from 10 to 83% mortality) and *Calliptamus barbarus* (from 40 to 100% mortality).

DISCUSSION

Several morphotypes exist in *B. bassiana* populations, each containing the strains with different levels of virulence. The morphological variants with salient and cottonlike colonies include most of the virulent isolates. These results differ somewhat from the earlier data on *B. bassiana* polymorphism [4, 6] and are in better accordance with the results on spontaneous variability in *M. anisopliae* [15]. However, selection for floury colonies without relief is practically more promising, since such cultures yield high conidial titers and biomass in a short time. Low correlation between virulence and morphophysiological characteristics of the isolates confirm that virulence is a result of numerous features of entomopathogenic micromycetes [1, 8, 16].

B. bassiana cultures isolated in a small territory or even at the same site from the same species of insects are highly variable in virulence. An isolate with high activity against insects of a certain order will quite probably be highly virulent to insects of other orders. No trophic specialization was revealed for the investigated isolates. These results are in accordance with the data of other authors. For example, the virulence of *B. bassiana* isolates to greenhouse whitefly *Trialeurodes vaporariorum* Wstw. was found to be independent of the source of isolation (various lepidopterans, dipterans, or *T. vaporariorum*) [4]. Investigation of genetic polymorphism in *B. bassiana* [2] revealed that territorial factors, rather than the host organisms, play the main role in intraspecific differentiation. The approach used in the present work is insufficient for outright denial of specialization to certain hosts in the populations of this fungus. Insect mortality is possibly not the criterion of *B. bassiana* specificity to different insect species. It was earlier demonstrated that not all hosts support the complete life cycle of different strains of filamentous fungi and result in abundant fruiting hyphae on dead insects [14]. Moreover, the "physiological" range of hosts determined under laboratory conditions may be different from the "ecological" range of hosts due to environment, behavior, characteristics of the host's life cycle, and other factors operating under natural conditions [17]. We also are not aware among which insect groups a strain circulated prior to its isolation from the terminal host. The issue of the possibility of formation of host-specialized races in a broadly specialized species *B. bassiana* requires investigation of the changes in the morphophysiological and virulent characteristics of the strains in a series of transfers in various hosts.

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